

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**



Office de la propriété  
intellectuelle  
du Canada

Un organisme  
d'Industrie Canada

Canadian  
Intellectual Property  
Office

An Agency of  
Industry Canada

*Bureau canadien  
des brevets  
Certification*

*Canadian Patent  
Office  
Certification*

La présente atteste que les documents  
ci-joints, dont la liste figure ci-dessous,  
sont des copies authentiques des docu-  
ments déposés au Bureau des brevets.

This is to certify that the documents  
attached hereto and identified below are  
true copies of the documents on file in  
the Patent Office.

Specification as originally filed, with Application for Patent Serial No: 2,305,036, on  
April 12, 2000, by **THE UNIVERSITY OF BRITISH COLUMBIA AND  
CHEMOKINE THERAPEUTICS CORPORATION**, assignee of Hassan Salari,  
Ahmed Merzouk, Geeta Saxena, Connie Eaves, Johanne Cashman and Ian Clark-Lewis,  
for "CXCR4 Antagonist Treatment of Hematopoietic Cells".

*Brian Sullivan*

Agent certificateur/Certifying Officer

December 2, 2003

Date

Canada

(CIPO 68)  
04-09-02

OPIC  CIPO

**ABSTRACT**

In accordance with various aspects of the invention, CXCR4 agonists may be used to treat bone marrow progenitor or stem cells to reduce the susceptibility of the cells to cytotoxic agents. CXCR4 agonists may be used to treat bone marrow progenitor cells or stem cells to reduce the rate of cellular multiplication. CXCR4 agonists may be used to treat cancer in a mammal in conjunction with one or more cytotoxic agents. Cytotoxic agents may for example include chemotherapeutic agents or radiation. CXCR4 agonists may be used therapeutically to regulate bone marrow progenitor or stem cell growth in human diseases, such as cancer.

## **CXCR4 AGONIST TREATMENT OF HEMATOPOIETIC CELLS**

### **FIELD OF THE INVENTION**

In one aspect, the invention relates to therapeutic uses of chemokine receptor agonists, including peptide agonists of CXC chemokine receptor 4 (CXCR4) for use in the treatment of cancer.

### **BACKGROUND OF THE INVENTION**

Cytokines are soluble proteins secreted by a variety of cells including monocytes or lymphocytes that regulate immune responses. Chemokines are a superfamily of chemoattractant proteins. Chemokines regulate a variety of biological responses and they promote the recruitment of multiple lineages of leukocytes and lymphocytes to a body organ tissue. Chemokines may be classified into two families according to the relative position of the first two cysteine residues in the protein. In one family, the first two cysteines are separated by one amino acid residue, the CXC chemokines, and in the other family the first two cysteines are adjacent, the CC chemokines. Two minor subgroups contain only one of the two cysteines (C) or have three amino acids between the cysteines (CX<sub>3</sub>C). In humans, the genes of the CXC chemokines are clustered on chromosome 4 (with the exception of SDF-1 gene, which has been localized to chromosome 10) and those of the CC chemokines on chromosome 17.

The molecular targets for chemokines are cell surface receptors. One such receptor is CXC chemokine receptor 4 (CXCR4), which is a 7 transmembrane protein, coupled to G1 and was previously called LESTR (Loetscher, M., Gelsner, T., O'Reilly, T., Zwaalen, R., Baggiolini, M., and Moser, B., (1994) J. Biol. Chem, 269, 232-237), HUMSTR (Federspiel, B., Duncan, A.M.V., Delaney, A., Schappert, K., Clark-Lewis, I., and Jirik, F.R. (1993) Genomics 16, 707-712) and Fusin (Feng, Y., Broeder, C.C., Kennedy, P.E., and Berger, E.A. (1996) HIV-1 entry cofactor: Functional cDNA cloning of a seven-

transmembrane G protein-coupled receptor, *Science* 272, 872-877). CXCR4 is widely expressed on cells of hemopoietic origin, and is a major co-receptor with CD4<sup>+</sup> for human immunodeficiency virus 1 (HIV-1) (Feng, Y., Broeder, C.C., Kennedy, P.E., and Berger, E.A. (1996) HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane G protein-coupled receptor, *Science* 272, 872-877).

Stromal cell derived factor one (SDF-1) is a natural ligand for CXCR4. Stromal cell derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) and stromal cell derived factor-1 $\beta$  (SDF-1 $\beta$ ) are closely related members (together referred to herein as SDF-1). The native amino acid sequences of SDF-1 $\alpha$  and SDF-1 $\beta$  are known, as are the genomic sequences encoding these proteins (see U.S. Patent No. 5,563,048 issued 8 October 1996, and U.S. Patent No. 5,756,084 issued 26 May 1998).

SDF-1 is functionally distinct from other chemokines in that it is reported to have a fundamental role in the trafficking, export and homing of bone marrow progenitor cells (Aiuti, A., Webb, I.J., Bleul, C., Springer, T., and Gulerrez-Ramos, J.C., (1996) *J. Exp. Med.* 185, 111-120 and Nagasawa, T., Hirota, S., Tachibana, K., Takakura N., Nishikawa, S.-I., Kitamura, Y., Yoshida, N., Kikutani, H., and Kishimoto, T., (1996) *Nature* 382, 635-638). SDF-1 is also structurally distinct in that it has only about 22% amino acid sequence identity with other CXC chemokines (Bleul, C.C., Fuhlbrigge, R.C., Casasnovas, J.M., Aiuti, A., and Springer, T.A., (1996) *J. Exp. Med.* 184, 1101-1109). SDF-1 appears to be produced constitutively by several cell types, and particularly high levels are found in bone-marrow stromal cells (Shirozu, M., Nakano, T., Inazawa, J., Tashiro, K., Tada, H., Shinozaki, T., and Honjo, T., (1995) *Genomics*, 28, 495-500 and Bleul, C.C., Fuhlbrigge, R.C., Casasnovas, J.M., Aiuti, A., and Springer, T.A., (1996) *J. Exp. Med.* 184, 1101-1109). A basic physiological role for SDF-1 is implied by the high level of conservation of the SDF-1 sequence between species. *In vitro*, SDF-1 stimulates chemotaxis of a wide range of cells including monocytes and bone marrow derived progenitor cells (Aiuti, A., Webb, I.J., Bleul,

C., Springer, T., and Guierrez-Ramos, J.C., (1996) J. Exp. Med. 185, 111-120 and Bleul, C.C., Fuhlbrigge, R.C., Casasnovas, J.M., Aiuti, A., and Springer, T.A., (1996) J. Exp. Med. 184, 1101-1109). SDF-1 also stimulates a high percentage of resting and activated T-lymphocytes (Bleul, C.C., Fuhlbrigge, R.C., Casasnovas, J.M., Aiuti, A., and Springer, T.A., (1996) J. Exp. Med. 184, 1101-1109 and Campbell, J.J., Hendrick, J., Zlotnik, A., Siani, M.A., Thompson, D.A., and Butcher, E.C., (1998) Science, 279 381-383).

A variety of diseases require treatment with agents which are preferentially cytotoxic to dividing cells. Cancer cells, for example, may be targeted with cytotoxic doses of radiation or chemotherapeutic agents. A significant side-effect of this approach to cancer therapy is the pathological impact of such treatments on rapidly dividing normal cells. These normal cells may for example include hair follicles, mucosal cells and the hematopoietic cells, such as primitive bone marrow progenitor cells and stem cells. The indiscriminate destruction of hematopoietic stem, progenitor or precursor cells can lead to a reduction in normal mature blood cell counts, such as leukocytes and red blood cells. A major impact on mature cell numbers may be seen particularly with neutrophils (neutropaenia) and platelets (thrombocytopenia), cells which naturally have relatively short half-lives. A decrease in leukocyte count, with concomitant loss of immune system function, may increase a patient's risk of opportunistic infection. Neutropaenia resulting from chemotherapy may for example occur within two or three days of cytotoxic treatments, and may leave the patient vulnerable to infection for up to 2 weeks until the haematopoietic system has recovered sufficiently to regenerate neutrophil counts. A reduced leukocyte count (leukopenia) as a result of cancer therapy may become sufficiently serious that therapy must be interrupted to allow the white blood cell count to rebuild. Interruption of cancer therapy can in turn lead to survival of cancer cells, an increase in the incidence of drug resistance in cancer cells, and ultimately in cancer relapse. There is accordingly a need for therapeutic agents and

4  
APR 12 2000 0:40 PM FR WILSON/DA VINCE/DOE BELT TO 1013306410 1:50/00

treatments which facilitate the preservation of hematopoietic progenitor or stem cells in patients subject to treatment with cytotoxic agents.

Bone marrow transplantation has been used in the treatment of a variety of hematological, autoimmune and malignant diseases. In conjunction with bone marrow transplantation, *ex vivo* hematopoietic (bone marrow) cell culture may be used to expand the population of hematopoietic progenitor or stem cells. It may be desirable to purge an *ex vivo* hematopoietic cell culture of cancer cells with cytotoxic treatments, while preserving the viability of the hematopoietic progenitor or stem cells. There is accordingly a need for agents and methods which facilitate the preservation of hematopoietic progenitor or stem cells in *ex vivo* cell cultures exposed to cytotoxic agents.

A number of proteins have been identified as inhibitors of hematopoietic progenitor cell development, with potential therapeutic usefulness as inhibitors of hematopoietic cell multiplication: macrophage inflammatory protein 1-alpha (MIP-1-alpha) and LD78 (see U.S. Patent No. 5,856,301); the alpha globin chain of hemoglobin and beta globin chain of hemoglobin (see U.S. Patent No. 6,022,848); and, interferon gamma (see U.S. Patent No. 5,807,744).

#### **SUMMARY OF THE INVENTION**

In accordance with various aspects of the invention, CXCR4 agonists may be used to treat bone marrow progenitor or stem cells to reduce the susceptibility of the cells to cytotoxic agents. CXCR4 agonists may be used to treat bone marrow progenitor cells or stem cells to reduce the rate of cellular multiplication. CXCR4 agonists may be used to treat cancer in a mammal in conjunction with one or more cytotoxic agents. Cytotoxic agents may for example include chemotherapeutic agents or radiation. CXCR4 agonists may be used therapeutically to regulate bone marrow progenitor or stem cell growth in human diseases, such as cancer.

#### **DETAILED DESCRIPTION OF THE INVENTION**

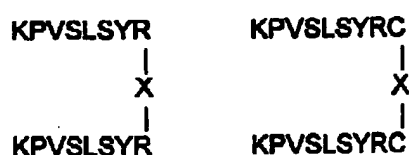
Cancers susceptible to treatment with CXCR4 agonists in accordance with various aspects of the invention may include both primary and metastatic solid tumors, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract, (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma) and tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas). In some aspects of the invention, CXCR4 agonists may also be useful in treating solid tumors arising from hematopoietic malignancies such as leukemias (i.e. chloromas, plasmacytomas and the plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia) as well as in the treatment of lymphomas (both Hodgkin's and non-Hodgkin's lymphomas). In addition, CXCR4 agonists may be useful in the prevention of metastases from the tumors described above either when used alone or in combination with cytotoxic agents such as radiotherapy or chemotherapeutic agents.

A variety of small SDF-1 peptide analogues may be used as CXCR4 agonists. One such peptide is a dimer of amino acids 1-9, in which the amino acid chains are joined by a disulphide bond between each of the cysteines at position 9 in each sequence (designated SDF-1(1-9)<sub>2</sub> or KPVSLSYRC-CRYSLSVPK). An alternative peptide is a dimer of amino acids 1-8, KPVSLSYR-X-RYSLSVPK, in which the amino acid chains are joined by a linking moiety X between each of the arginines at position 8 in each sequence (designated SDF-1(1-8)<sub>2</sub>). CXCR4 agonist peptides may for example be selected from the group



consisting of peptides having the following sequences:

KPVLSYRCPCRFFESHVARANVKHLKILNTPNCALQIVARLKNNNRQVCIDPKL  
KWIQEYLEKALN; KPVLSYRCPCRFFESH; KPVLSYRC; KPVLSYRC-  
CRYSLSVPK; KPVLSYRC-X-CRYSLSVPK; and, KPVLSYR-X-RYSLSVPK. In  
the foregoing peptides X may be lysine with both the  $\alpha$  and  $\epsilon$  amino groups of the  
lysine being associated with covalent (amide) bond formation and the lysyl  
carboxyl group being protected. The last two compounds in the foregoing list may,  
for example, be represented as follows, showing the peptide sequences in the  
standard amino-to-carboxyl orientation:



In some embodiments, the CXCR4 agonists for use in the invention may be substantially purified peptide fragments, modified peptide fragments, analogues or pharmacologically acceptable salts of either SDF-1 $\alpha$  or SDF-1 $\beta$ . SDF-1 derived peptide agonists of CXCR4 may be identified by known physiological assays and a variety of synthetic techniques (such as disclosed in Crump et al., 1997, The EMBO Journal 16(23) 6996-7007; and Heveker et al., 1998, Current Biology 8(7): 369-376; each of which are incorporated herein by reference). Such SDF-1 derived peptides may include homologs of native SDF-1, such as naturally occurring isoforms or genetic variants, or polypeptides having substantial sequence similarity to SDF-1, such as 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% sequence identity to at least a portion of the native SDF-1 sequence, the portion of native SDF-1 being any contiguous sequence of 10, 20, 30, 40, 50 or more amino acids, provided the peptides have CXCR4 agonist activity. In some embodiments, chemically similar amino acids may be substituted for amino acids in the native SDF-1 sequence (to provide conservative amino acid substitutions). In some embodiments, peptides having

an N-terminal LSY sequence motif within 10, or 7, amino acids of the N-terminus, and/or an N-terminal RFFESH (SEQ ID NO:5) sequence motif within 20 amino acids of the N-terminus may be used provided they have CXCR4 agonistic activity. One family of such peptide agonist candidates has an LSY motif at amino acids 5-7. Alternative peptides further include the RFFESH (SEQ ID NO: 5) motif at amino acids 12-17. In alternative embodiments, the LSY motif is located at positions 3-5 of a peptide. The invention also provides peptide dimers having two amino acid sequences, which may each have the foregoing sequence elements, attached by a disulfide bridge within 20, or preferably within 10, amino acids of the N terminus, linking cysteine residues or  $\alpha$ -aminobutyric acid residues.

The invention provides pharmaceutical compositions containing CXCR4 agonists. In one embodiment, such compositions include a CXCR4 agonist compound in a therapeutically or prophylactically effective amount sufficient to alter bone marrow progenitor or stem cell growth, and a pharmaceutically acceptable carrier. In another embodiment, the composition includes a CXCR4 agonist compound in a therapeutically or prophylactically effective amount sufficient to inhibit a cytotoxic effect of a cytotoxic agent, such as cytotoxic agents used in chemotherapy or radiation treatment of cancer, and a pharmaceutically acceptable carrier.

A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as reduction of bone marrow progenitor or stem cell multiplication, or reduction or inhibition of a cytotoxic effect of a cytotoxic agent. A therapeutically effective amount of CXCR4 agonist may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the CXCR4 agonist to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the CXCR4 agonist are outweighed by the therapeutically beneficial effects.

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as preventing or inhibiting a cytotoxic effect of a cytotoxic agent. Typically, a prophylactic dose is used in subjects prior to or at an earlier stage of disease, so that a prophylactically effective amount may be less than a therapeutically effective amount.

In particular embodiments, a preferred range for therapeutically or prophylactically effective amounts of CXCR4 agonists may be 0.1 nM-0.1M, 0.1 nM-0.05M, 0.05 nM-15µM or 0.01 nM-10 M. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions. Dosage ranges set forth herein are exemplary only and do not limit the dosage ranges that may be selected by medical practitioners.

The amount of active compound in the composition may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique

characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Alternatively, the carrier can be suitable for intravenous, intraperitoneal, intramuscular, sublingual or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an

CXCR4 antagonist compounds of the invention may include SDF-1 derivatives, such as C-terminal hydroxymethyl derivatives, O-modified derivatives (e.g., C-terminal hydroxymethyl benzyl ether), N-terminally modified derivatives including substituted amides such as alkylamides and hydrazides and compounds in which a C-terminal phenylalanine residue is replaced with a phenethylamide analogue (e.g., Ser-Ile-phenethylamide as an analogue of the tripeptide Ser-Ile-Phe).

Within a CXCR4 agonist compound of the invention, a peptidic structure (such as an SDF-1 derived peptide) maybe coupled directly or indirectly to at least one modifying group. The term "modifying group" is intended to include structures that are directly attached to the peptidic structure (e.g., by covalent coupling), as well as those that are indirectly attached to the peptidic structure (e.g., by a stable non-covalent association or by covalent coupling to additional amino acid residues, or mimetics, analogues or derivatives thereof, which may flank the SDF-1 core peptidic structure). For example, the modifying group can be coupled to the amino-terminus or carboxy-terminus of an SDF-1 peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain. Alternatively, the modifying group can be coupled to a side chain of at least one amino acid residue of a SDF-1 peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain (e.g., through the epsilon amino group of a lysyl residue(s), through the carboxyl group of an aspartic acid residue(s) or a glutamic acid residue(s), through a hydroxy group of a tyrosyl residue(s), a serine residue(s) or a threonine residue(s) or other suitable reactive group on an amino acid side chain). Modifying groups covalently coupled to the peptidic structure can be attached by means and using methods well known in the art for linking chemical structures, including, for example, amide, alkylamino, carbamate or urea bonds.

In some embodiments, the modifying group may comprise a cyclic, heterocyclic or polycyclic group. The term "cyclic group", as used herein, includes cyclic saturated or unsaturated (i.e., aromatic) group having from 3 to 10, 4 to 8, or 6 to 7 carbon atoms. Exemplary cyclic groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclooctyl. Cyclic groups may be unsubstituted or substituted at one or more ring positions. A cyclic group may for example be substituted with halogens, alkyls, cycloalkyls, alkenyls, alkynyls, aryls, heterocycles, hydroxyls, amines, nitros, thiols amines, imines, amides,

phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, sulfonates, selenoethers, ketones, aldehydes, esters, -CF<sub>3</sub>, -CN.

The term "heterocyclic group" includes cyclic saturated, unsaturated and aromatic groups having from 3 to 10, 4 to 8, or 5 to 7 carbon atoms, wherein the ring structure includes about one or more heteroatoms. Heterocyclic groups include pyrrolidine, oxolane, thiolane, imidazole, oxazole, piperidine, piperazine, morpholine. The heterocyclic ring may be substituted at one or more positions with such substituents as, for example, halogens, alkyls, cycloalkyls, alkenyls, alkynyls, aryls, other heterocycles, hydroxyl, amino, nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, -CF<sub>3</sub>, -CN. Heterocycles may also be bridged or fused to other cyclic groups as described below.

The term "polycyclic group" as used herein is intended to refer to two or more saturated, unsaturated or aromatic cyclic rings in which two or more carbons are common to two adjoining rings, so that the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycyclic group may be substituted with such substituents as described above, as for example, halogens, alkyls, cycloalkyls, alkenyls, alkynyls, hydroxyl, amino, nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, -CF<sub>3</sub>, or -CN.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In some embodiments, a straight chain or branched chain alkyl has 20 or fewer carbon atoms in its backbone (C<sub>1</sub>-C<sub>20</sub> for straight chain, C<sub>3</sub>-C<sub>20</sub> for branched chain), or 10 or fewer carbon atoms. In some embodiments,

cycloalkyls may have from 4-10 carbon atoms in their ring structure, such as 5, 6 or 7 carbon rings. Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, having from one to ten carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have chain lengths of ten or less carbons.

The term "alkyl" (or "lower alkyl") as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, carbonyl (such as carboxyl, ketones (including alkylcarbonyl and arylcarbonyl groups), and esters (including alkylloxycarbonyl and aryloxycarbonyl groups)), thiocarbonyl, acyloxy, alkoxyl, phosphoryl, phosphonate, phosphinate, amino, acylamino, amido, amidine, imino, cyano, nitro, azido, sulfhydryl, alkylthio, sulfate, sulfonate, sulfamoyl, sulfonamido, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety. The moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of aminos, azidos, iminos, amidos, phosphoryls (including phosphonates and phosphinates), sulfonyls (including sulfates, sulfonamidos, sulfamoyls and sulfonates), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), -CF<sub>3</sub>, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, -CF<sub>3</sub>, -CN, and the like.

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.



The term "aralkyl", as used herein, refers to an alkyl or alkenyl group substituted with at least one aryl group. Exemplary aralkyls include benzyl (i.e., phenylmethyl), 2-naphthylethyl, 2-(2-pyridyl)propyl, 5-dibenzosuberonyl, and the like.

The term "alkylcarbonyl", as used herein, refers to  $-C(O)-alkyl$ . Similarly, the term "arylcarbonyl" refers to  $-C(O)-aryl$ . The term "alkyloxycarbonyl", as used herein, refers to the group  $-C(O)-O-alkyl$ , and the term "aryloxycarbonyl" refers to  $-C(O)-O-aryl$ . The term "acyloxy" refers to  $-O-C(O)-R_7$ , in which  $R_7$  is alkyl, alkenyl, alkynyl, aryl, aralkyl or heterocyclyl.

The term "amino", as used herein, refers to  $-N(R_a)(R_b)$ , in which  $R_a$  and  $R_b$  are each independently hydrogen, alkyl, alkenyl, alkynyl, aralkyl, aryl, or in which  $R_a$  and  $R_b$  together with the nitrogen atom to which they are attached form a ring having 4-8 atoms. Thus, the term "amino", as used herein, includes unsubstituted, monosubstituted (e.g., monoalkylamino or monoarylamino), and disubstituted (e.g., dialkylamino or alkylarylamino) amino groups. The term "amido" refers to  $-C(O)-N(R_a)(R_b)$ , in which  $R_a$  and  $R_b$  are as defined above. The term "acylamino" refers to  $-N(R'_a)C(O)-R_7$ , in which  $R_7$  is as defined above and  $R'_a$  is alkyl.

As used herein, the term "nitro" means  $-NO_2$ ; the term "halogen" designates  $-F$ ,  $-Cl$ ,  $-Br$  or  $-I$ ; the term "sulfhydryl" means  $-SH$ ; and the term "hydroxyl" means  $-OH$ .

The term "aryl" as used herein includes 5-, 6- and 7-membered aromatic groups that may include from zero to four heteroatoms in the ring, for example, phenyl, pyrrolyl, furyl, thiophenyl, imidazolyl, oxazole, thiazolyl, triazolyl, pyrazolyl, pyridyl, pyrazinyl, pyridazinyl and pyrimidinyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics". The aromatic ring can be substituted at one

example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF<sub>3</sub>, -CN, or the like. Aryl groups can also be part of a polycyclic group. For example, aryl groups include fused aromatic moieties such as naphthyl, anthracenyl, quinolyl, indolyl, and the like.

Modifying groups may include groups comprising biotinyl structures, fluorescein-containing groups, a diethylene-triaminepentaacetyl group, a (-)-menthoxyacetyl group, a N-acetylneuraminyl group, a cholyl structure or an iminobiotinyl group. A CXCR4 agonist compound may be modified at its carboxy terminus with a cholyl group according to methods known in the art (see e.g., Wess, G. et al. (1993) *Tetrahedron Letters*, 34:817-822; Wess, G. et al. (1992) *Tetrahedron Letters* 33:195-198; and Kramer, W. et al. (1992) *J. Biol. Chem.* 267:18598-18604). Cholyl derivatives and analogues may also be used as modifying groups. For example, a preferred cholyl derivative is Alc (3-(O-aminoethyl-iso)-cholyl), which has a free amino group that can be used to further modify the CXCR4 agonist compound. A modifying group may be a "biotinyl structure", which includes biotinyl groups and analogues and derivatives thereof (such as a 2-iminobiotinyl group). In another embodiment, the modifying group may comprise a "fluorescein-containing group", such as a group derived from reacting an SDF-1 derived peptidic structure with 5-(and 6)-carboxyfluorescein, succinimidyl ester or fluorescein isothiocyanate. In various other embodiments, the modifying group(s) may comprise an N-acetylneuraminyl group, a trans-4-cotininecarboxyl group, a 2-imino-1-imidazolidineacetyl group, an (S)-(-)-indoline-2-carboxyl group, a (-)-menthoxyacetyl group, a 2-norbornaneacetyl group, a 2-oxo-5-acenaphthenebutyryl, a (-)-2-oxo-4-thiazolidinecarboxyl group, a tetrahydro-3-furoyl group, a 2-iminobiotinyl group, a

diethylenetriaminepentaacetyl group, a 4-morpholinecarbonyl group, a 2-thiophenecarboxyl group or a 2-thiophenesulfonyl group.

A CXCR4 agonist compound of the invention may be further modified to alter the specific properties of the compound while retaining the desired functionality of the compound. For example, in one embodiment, the compound may be modified to alter a pharmacokinetic property of the compound, such as in vivo stability or half-life. The compound may be modified to label the compound with a detectable substance. The compound may be modified to couple the compound to an additional therapeutic moiety. To further chemically modify the compound, such as to alter its pharmacokinetic properties, reactive groups can be derivatized. For example, when the modifying group is attached to the amino-terminal end of the SDF-1 core domain, the carboxy-terminal end of the compound may be further modified. Potential C-terminal modifications include those which reduce the ability of the compound to act as a substrate for carboxypeptidases. Examples of C-terminal modifiers include an amide group, an ethylamide group and various non-natural amino acids, such as D-amino acids and -alanine. Alternatively, when the modifying group is attached to the carboxy-terminal end of the aggregation core domain, the amino-terminal end of the compound may be further modified, for example, to reduce the ability of the compound to act as a substrate for aminopeptidases.

A CXCR4 agonist compound can be further modified to label the compound by reacting the compound with a detectable substance. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or

phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include  $^{14}\text{C}$ ,  $^{123}\text{I}$ ,  $^{124}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ . A CXCR4 agonist compound may be radioactively labeled with  $^{14}\text{C}$ , either by incorporation of  $^{14}\text{C}$  into the modifying group or one or more amino acid structures in the CXCR4 agonist compound. Labeled CXCR4 agonist compounds may be used to assess the *in vivo* pharmacokinetics of the compounds, as well as to detect disease progression or propensity of a subject to develop a disease, for example for diagnostic purposes. Tissue distribution CXCR4 receptors can be detected using a labeled CXCR4 agonist compound either *in vivo* or in an *in vitro* sample derived from a subject. For use as an *in vivo* diagnostic agent, a CXCR4 antagonist compound of the invention may be labeled with radioactive technetium or iodine. A modifying group can be chosen that provides a site at which a chelation group for the label can be introduced, such as the Alc derivative of cholic acid, which has a free amino group. For example, a phenylalanine residue within the SDF-1 sequence (such as amino acid residue 13) may be substituted with radioactive iodotyrosyl. Any of the various isotopes of radioactive iodine may be incorporated to create a diagnostic agent.  $^{123}\text{I}$  (half-life=13.2 hours) may be used for whole body scintigraphy,  $^{124}\text{I}$  (half life=4 days) may be used for positron emission tomography (PET),  $^{125}\text{I}$  (half life=60 days) may be used for metabolic turnover studies and  $^{131}\text{I}$  (half life=8 days) may be used for whole body counting and delayed low resolution imaging studies.

In an alternative chemical modification, a CXCR4 agonist compound of the invention may be prepared in a "prodrug" form, wherein the compound itself does not act as a CXCR4 agonist, but rather is capable of being transformed, upon metabolism *in vivo*, into a CXCR4 agonist compound as defined herein. For example, in this type of compound, the modifying group can be present in a prodrug form that is capable of being converted upon metabolism into the form of an active CXCR4 agonist. Such a prodrug form of a modifying group is referred to herein as a "secondary modifying group." A variety of strategies are known in the art for preparing peptide prodrugs that limit metabolism in order to optimize

delivery of the active form of the peptide-based drug (see e.g., Moss, J. (1995) in *Peptide-Based Drug Design: Controlling Transport and Metabolism*, Taylor, M. D. and Amidon, G. L. (eds), Chapter 18.

CXCR4 agonist compounds of the invention may be prepared by standard techniques known in the art. A peptide component of a CXCR4 agonist may be composed, at least in part, of a peptide synthesized using standard techniques (such as those described in Bodansky, M. *Principles of Peptide Synthesis*, Springer Verlag, Berlin (1993); Grant, G. A. (ed.), *Synthetic Peptides: A User's Guide*, W. H. Freeman and Company, New York (1992); or Clark-Lewis, I., Dewald, B., Loetscher, M., Moser, B., and Baggiolini, M., (1994) *J. Biol. Chem.*, 269, 16075-16081). Automated peptide synthesizers are commercially available (e.g., Advanced ChemTech Model 396; Milligen/Bioscience 9600). Peptides may be assayed for CXCR4 agonist activity in accordance with standard methods. Peptides may be purified by HPLC and analyzed by mass spectrometry. Peptides may be dimerized via a disulfide bridge formed by gentle oxidation of the cysteines using 10% DMSO in water. Following HPLC purification dimer formation may be verified, by mass spectrometry. One or more modifying groups may be attached to a SDF-1 derived peptidic component by standard methods, for example using methods for reaction through an amino group (e.g., the alpha-amino group at the amino-terminus of a peptide), a carboxyl group (e.g., at the carboxy terminus of a peptide), a hydroxyl group (e.g., on a tyrosine, serine or threonine residue) or other suitable reactive group on an amino acid side chain (see e.g., Greene, T. W. and Wuts, P. G. M. *Protective Groups in Organic Synthesis*, John Wiley and Sons, Inc., New York (1991)).

In another aspect of the invention, CXCR4 agonist peptides may be prepared according to standard recombinant DNA techniques using a nucleic acid molecule encoding the peptide. A nucleotide sequence encoding the peptide may be determined using the genetic code and an oligonucleotide molecule having this nucleotide sequence may be synthesized by standard DNA synthesis

methods (e.g., using an automated DNA synthesizer). Alternatively, a DNA molecule encoding a peptide compound may be derived from the natural precursor protein gene or cDNA (e.g., using the polymerase chain reaction (PCR) and/or restriction enzyme digestion) according to standard molecular biology techniques.

The invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a peptide of the invention. In some embodiments, the peptide may comprise an amino acid sequence having at least one amino acid deletion compared to native SDF-1. The term "nucleic acid molecule" is intended to include DNA molecules and RNA molecules and may be single-stranded or double-stranded. In alternative embodiments, the isolated nucleic acid encodes a peptide wherein one or more amino acids are deleted from the N-terminus, C-terminus and/or an internal site of SDF-1.

To facilitate expression of a peptide compound in a host cell by standard recombinant DNA techniques, the isolated nucleic acid encoding the peptide may be incorporated into a recombinant expression vector. Accordingly, the invention also provides recombinant expression vectors comprising the nucleic acid molecules of the invention. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been operatively linked. Vectors may include circular double stranded DNA plasmids, viral vectors. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (such as bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (such as non-episomal mammalian vectors) may be integrated into the genome of a host cell upon introduction into the host cell, and thereby may be replicated along with the host genome. Certain vectors may be capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" or "expression vectors".

In recombinant expression vectors of the invention, the nucleotide sequence encoding a peptide may be operatively linked to one or more regulatory sequences, selected on the basis of the host cells to be used for expression. The terms "operatively linked" or "operably" linked mean that the sequences encoding the peptide are linked to the regulatory sequence(s) in a manner that allows for expression of the peptide compound. The term "regulatory sequence" includes promoters, enhancers, polyadenylation signals and other expression control elements. Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, Calif. (1990) (incorporated herein by reference). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell, those that direct expression of the nucleotide sequence only in certain host cells (such as tissue-specific regulatory sequences) and those that direct expression in a regulatable manner (such as only in the presence of an inducing agent). The design of the expression vector may depend on such factors as the choice of the host cell to be transformed and the level of expression of peptide compound desired.

The recombinant expression vectors of the invention may be designed for expression of peptide compounds in prokaryotic or eukaryotic cells. For example, peptide compounds may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector may be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari et al., (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, Calif.). Baculovirus vectors available for expression of proteins or peptides in cultured insect cells

(e.g., Sf 9 cells) include the pAc series (Smith et al., (1983) Mol. Cell. Biol. 3:2166-2165) and the pVL series (Lucklow, V. A., and Summers, M. D., (1989) Virology 170:31-39). Examples of mammalian expression vectors include pCDM8 (Seed, B., (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987), EMBO J. 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In addition to regulatory control sequences, recombinant expression vectors may contain additional nucleotide sequences, such as a selectable marker gene to identify host cells that have incorporated the vector. Selectable marker genes are well known in the art. To facilitate secretion of the peptide compound from a host cell, in particular mammalian host cells, the recombinant expression vector preferably encodes a signal sequence operatively linked to sequences encoding the amino-terminus of the peptide compound, such that upon expression, the peptide compound is synthesised with the signal sequence fused to its amino terminus. This signal sequence directs the peptide compound into the secretory pathway of the cell and is then cleaved, allowing for release of the mature peptide compound (i.e., the peptide compound without the signal sequence) from the host cell. Use of a signal sequence to facilitate secretion of proteins or peptides from mammalian host cells is well known in the art.

A recombinant expression vector comprising a nucleic acid encoding a peptide compound may be introduced into a host cell to produce the peptide compound in the host cell. Accordingly, the invention also provides host cells containing the recombinant expression vectors of the invention. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny



may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell may be any prokaryotic or eukaryotic cell. For example, a peptide compound may be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells. The peptide compound may be expressed *in vivo* in a subject to the subject by gene therapy (discussed further below).

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms "transformation" and "transfection" refer to techniques for introducing foreign nucleic acid into a host cell, including calcium phosphate or calcium chloride coprecipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, microinjection and viral-mediated transfection. Suitable methods for transforming or transfecting host cells can for example be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory manuals. Methods for introducing DNA into mammalian cells *in vivo* are also known, and may be used to deliver the vector DNA of the invention to a subject for gene therapy.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (such as resistance to antibiotics) may be introduced into the host cells along with the gene of interest. Preferred selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acids encoding a selectable marker may be introduced into a host cell on the same vector as that encoding the peptide compound or may be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid may be identified by drug selection (cells that have incorporated the selectable marker gene will survive, while the other cells die).

A nucleic acid of the invention may be delivered to cells *in vivo* using methods such as direct injection of DNA, receptor-mediated DNA uptake or viral-mediated transfection. Direct injection has been used to introduce naked DNA into cells *in vivo* (see e.g., Acsadi et al. (1991) *Nature* 332:815-818; Wolff et al. (1990) *Science* 247:1465-1468). A delivery apparatus (e.g., a "gene gun") for injecting DNA into cells *in vivo* may be used. Such an apparatus may be commercially available (e.g., from BioRad). Naked DNA may also be introduced into cells by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor (see for example Wu, G. and Wu, C. H. (1988) *J. Biol. Chem.* 263:14621; Wilson et al. (1992) *J. Biol. Chem.* 267:963-967; and U.S. Pat. No. 5,166,320). Binding of the DNA-ligand complex to the receptor may facilitate uptake of the DNA by receptor-mediated endocytosis. A DNA-ligand complex linked to adenovirus capsids which disrupt endosomes, thereby releasing material into the cytoplasm, may be used to avoid degradation of the complex by intracellular lysosomes (see for example Curiel et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8850; Cristiano et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:2122-2126).

Defective retroviruses are well characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A. D. (1990) *Blood* 76:271). Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in *Current Protocols in Molecular Biology*, Ausubel, F. M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines include p i.Crip, p i.Cre, p i.2 and p i.Am. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, endothelial cells, lymphocytes, myoblasts, hepatocytes, bone marrow cells, *in vitro* and/or *in vivo* (see for example Egltis, et al. (1985) *Science* 230:1395-1398; Danos and Mulligan

(1988) Proc. Natl. Acad. Sci. USA 85:6460-6464; Wilson et al. (1988) Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano et al. (1990) Proc. Natl. Acad. Sci. USA 87:6141-6145; Huber et al. (1991) Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry et al. (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury et al. (1991) Science 254:1802-1805; van Beusechem et al. (1992) Proc. Natl. Acad. Sci. USA 89:7640-7644; Kay et al. (1992) Human Gene Therapy 3:641-647; Dai et al. (1992) Proc. Natl. Acad. Sci. USA 89:10892-10895; Hwu et al. (1993) J. Immunol. 150:4104-4115; U.S. Pat. No. 4,888,118; U.S. Pat. No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573).

The genome of an adenovirus may be manipulated so that it encodes and expresses a peptide compound of the invention, but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See for example Berkner et al. (1988) BioTechniques 6:616; Rosenfeld et al. (1991) Science 252:431-434; and Rosenfeld et al. (1992) Cell 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Recombinant adenoviruses are advantageous in that they do not require dividing cells to be effective gene delivery vehicles and can be used to infect a wide variety of cell types, including airway epithelium (Rosenfeld et al. (1992) cited supra), endothelial cells (Lemarchand et al. (1992) Proc. Natl. Acad. Sci. USA 89:6482-6486), hepatocytes (Herz and Gerard (1993) Proc. Natl. Acad. Sci. USA 90:2812-2816) and muscle cells (Quantin et al. (1992) Proc. Natl. Acad. Sci. USA 89:2581-2584).

Adeno-associated virus (AAV) may be used for delivery of DNA for gene therapy purposes. AAV is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle (Muzyczka et al. Curr. Topics in Micro. and Immunol. (1992) 158:97-129). AAV may be used to integrate DNA

into non-dividing cells (see for example Flotte et al. (1992) Am. J. Respir. Cell. Mol. Biol. 7:349-356; Samulski et al. (1989) J. Virol. 63:3822-3828; and McLaughlin et al. (1989) J. Virol. 62:1963-1973). An AAV vector such as that described in Tratschin et al. (1985) Mol. Cell. Biol. 5:3251-3260 may be used to introduce DNA into cells (see for example Hermonat et al. (1984) Proc. Natl. Acad. Sci. USA 81:6466-6470; Tratschin et al. (1985) Mol. Cell. Biol. 4:2072-2081; Wondisford et al. (1988) Mol. Endocrinol. 2:32-39; Tratschin et al. (1984) J. Virol. 51:611-619; and Flotte et al. (1993) J. Biol. Chem. 268:3781-3790).

General methods for gene therapy are known in the art. See for example, U.S. Pat. No. 5,399,346 by Anderson et al. A biocompatible capsule for delivering genetic material is described in PCT Publication WO 95/05452 by Baetge et al. Methods for grafting genetically modified cells to treat central nervous system disorders are described in U.S. Pat. No. 5,082,670 and in PCT Publications WO 90/06757 and WO 93/10234, all by Gage et al.

Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. In the claims, the word "comprising" is used as an open-ended term, substantially equivalent to the phrase "including, but not limited to".

#### **Example 1**

Table 1 shows the effect of CXCR4 agonists on bone marrow progenitor cells, particularly primitive erythroid cells and primitive granulocytes, compared to mature granulocytes. To obtain the data in Table 1, cells were pre-incubated with each of the compounds or saline alone ('no drug' as control). The cells were then exposed to high dose H<sup>3</sup>-thymidine, a cytotoxic agent. Rapidly dividing cells

accumulate proportionally more of the cytotoxic radioactive thymidine and as a result are preferentially killed. The relative proportion of cells killed by the thymidine treatment compared to the control is indicative of the relative effectiveness of the compounds in reducing cellular multiplication, *i.e.* decreasing the rate of cell cycle progression. A higher (or unchanged) proportion of killed cells compared to the control is indicative that a compound does not reduce cellular multiplication of the given cell type.

**Table 1:**  
**Effect of CXCR4 Agonists on Bone Marrow Progenitor Cells Exposed to H<sup>3</sup>-Thymidine.**

	% CELL KILLED		
	No drug (control)	Compound #1	Compound #2
Primitive Erythroide	71	2	9
Primitive Granulocyte	48	1	1
Mature Granulocyte	39	45	42

In Table 1, Compound #1 is the peptide  
 KPVSLSYRCPCRFFESHVARANVKHLKILNTPNCALQIVARLKNNNRQVCIDPKL  
 KWIQEYLEKALN, used at 100 ng/ml on a human bone marrow cell culture.  
 Compound #2 is the peptide KPVSLSYRC-X-CRYSLSVPK (SDF-1(1-9)<sub>2</sub>), used  
 at 50 ug/ml on a human bone marrow cell culture.

**WHAT IS CLAIMED IS:**

1. A method of reducing the rate of hematopoietic cell multiplication, comprising administering an effective amount of a CXCR4 agonist to cells selected from the group consisting of hematopoietic stem cells and hematopoietic progenitor cells.
2. The method of claim 1, wherein the cells are *in vivo* in a patient and a therapeutically effective amount of the CXCR4 agonist is administered to the patient in need of such treatment.
3. The method of claim 2, wherein the patient has a cancer.
4. The method of claim 2 or 3, further comprising treating the patient with a cytotoxic agent, wherein the effective amount of the CXCR4 agonist is sufficient to reduce the susceptibility of the cells to the cytotoxic agent.
5. The method of any one of claims 1 through 4, wherein the CXCR4 agonist comprises a peptide.
6. The method of claim 5, wherein the peptide is selected from the group consisting of peptides having the sequence of:  
KPVLSYRCPCRFFESHVARANVKHLKILNTPNCALQIVARLKN  
NRQVCIDPKLKWIQEYLEKALN; KPVLSYRCPCRFFESH;  
KPVLSYRC; KPVLSYRC-X-CRYLSVVPK; and, KPVLSYR-X-  
RYSLSVVPK.
7. A method of reducing the susceptibility of cells to a cytotoxic agent, comprising treating a patient in need of such treatment with a therapeutically effective amount of a CXCR4 agonist, wherein the

cells are selected from the group consisting of hematopoietic stem cells and hematopoietic progenitor cells.

8. The use of a CXCR4 agonist to reduce the rate of multiplication of cells selected from the group consisting of hematopoietic stem cells and hematopoietic progenitor cells..
9. The use of a CXCR4 agonist to reduce the susceptibility cells to a cytotoxic agent, wherein the cells are selected from the group consisting of hematopoietic stem cells and hematopoietic progenitor cells..